



Development of a new cultivar with high yield and high-temperature tolerance by crossbreeding of *Undaria pinnatifida* (Laminariales, Phaeophyta)



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ABSTRACT

Recent production of the marine crop *Undaria pinnatifida* has become unstable due to rising temperatures caused by global climate change. The cultivar HGU-1 was bred in a previous study to achieve early cultivation. A new cultivar, NW-1, was bred for improved yield and stress tolerance. This was achieved by crossbreeding using male and female gametophytes, each isolated from a single zoospore. To evaluate whether the cultivar NW-1 is superior to the previous cultivar HGU-1, we compared their growth characteristics in an *Undaria* farm, in the eastern Seto Inland Sea of Japan. Despite the early start of nursery cultivation, due to a high seawater temperature of 24.5 °C, many young sporophytes of NW-1 grew on strings wound around the collector, whereas most of the HGU-1 sporophytes fell from the strings. After nursery cultivation, seawater temperature during rope cultivation was remarkably higher than usual. Nevertheless, the mean total length and weight of NW-1 were 115.5 cm and 140.1 g, respectively, at the end of December. In contrast, the mean total length and weight of HGU-1, at the corresponding time, were 85.4 cm and 58.1 g, respectively. Moreover, the growth and weight of the NW-1 sporophytes were significantly higher than those of HGU-1 throughout experimental cultivation. These results demonstrate that NW-1 is an excellent cultivar with a high yield and high-temperature tolerance.

1. Introduction

The kelp *Undaria pinnatifida* (Harvey) Suringar has a heteromorphic life cycle with macroscopic diploid sporophytes and microscopic haploid gametophytes. It is extensively cultivated as it is one of the most valuable edible seaweeds in Japan, Korea, and China (Akiyama and Kurogi, 1982; Sohn, 1993; Tseng, 2001). In addition, commercial cultivation of the marine crop is being developed in Spain (Peteiro and Freire, 2011). In Japan, total production value of the marine crop amounted to approximately \$US 90 million in 2016 according to the Ministry of Agriculture, Forestry and Fisheries, Japan. It is the second highest value for seaweeds cultivated in Japan, behind the red alga *Pyropia yezoensis* (nori). The Sanriku coast of north Japan and the eastern Seto Inland Sea, including Naruto and Awaji Island, are famous for Japanese *Undaria* cultivation. The cultivation season usually starts from autumn and runs through to spring. However, the start of the cultivation period has been delayed due to the influence of rising temperatures caused by global climate change. In addition, this prompted many germlings (juvenile sporophytes) of *U. pinnatifida* to

fall from the strings during nursery cultivation. Moreover, the growth of young sporophytes cultivated on ropes is also slower than previously, because of the high seawater temperature. Furthermore, bleaching of *Undaria* sporophytes (i.e., decrease of photosynthetic pigments) has been caused recently due to nitrogen depletion in the eastern Seto Inland Sea and has caused economic damage to *Undaria* and *Pyropia* (nori) cultivators (Nagata et al., 1998; Nishikawa et al., 2007; Niwa and Harada, 2013, 2016). Therefore, similar to cultivated *Pyropia*, the recent production of cultivated *U. pinnatifida* has also become unstable.

Due to the above-reasons, it needs new breeds which possess both a higher yield and higher temperature tolerance. If they can start the cultivation and harvest early (e.g., by forcing cultivation), the price of the marine crop increases. Thus, forced cultivation provides additional benefits. In the eastern Seto Inland Sea, the *Undaria* germlings cultured in water tanks are usually transferred to the sea for nursery cultivation when the seawater temperature is approximately 23 °C (Dan et al., 2015). However, the cultivar HGU-1, that was developed for forced cultivation by crossbreeding of wild and cultivated *U. pinnatifida*, enables *Undaria* cultivators to start the nursery cultivation at a seawater

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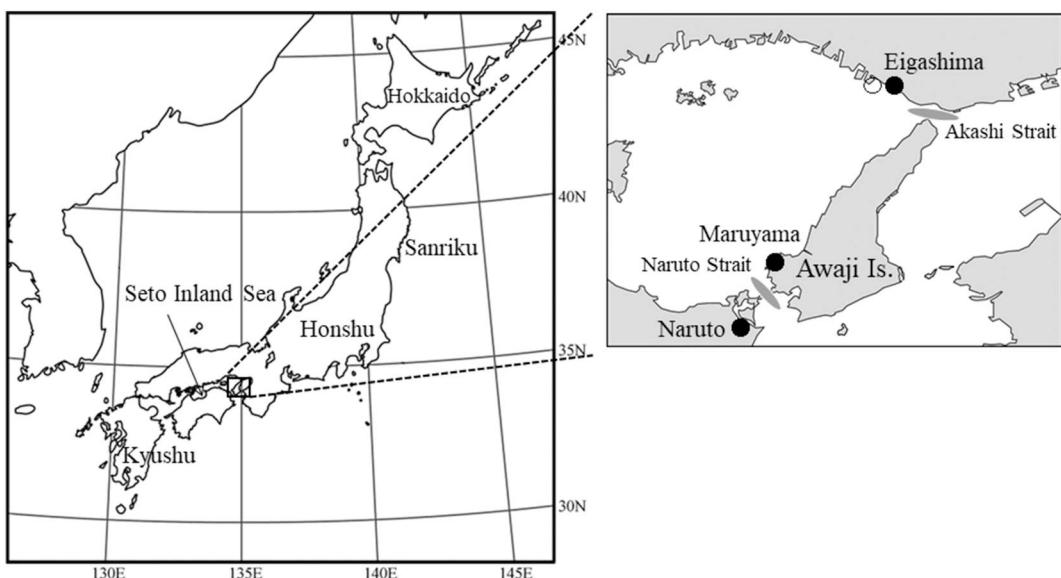


Fig. 1. Maps showing the cultivation site of Eigashima in the eastern Seto Inland Sea, and other localities mentioned in this study. The open circle shows the measurement site for seawater temperature.

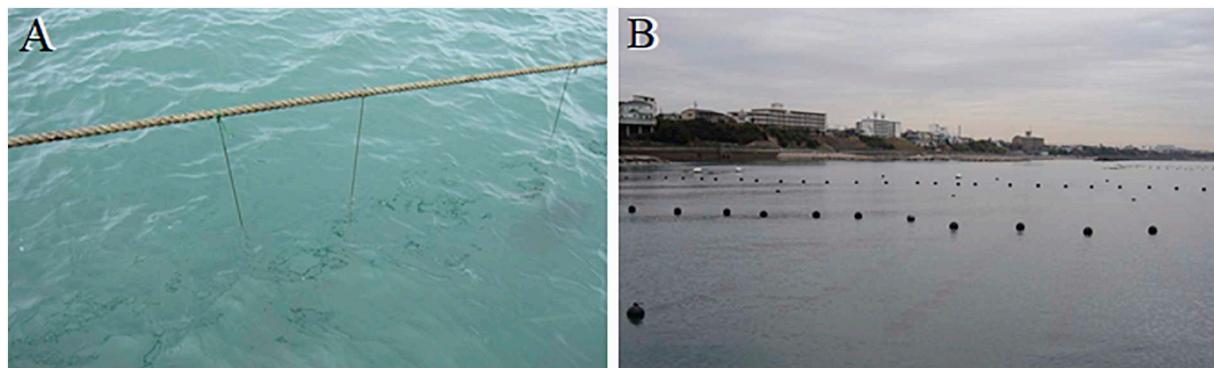


Fig. 2. Nursery cultivation (A) and cultivation for harvest (B) of *Undaria pinnatifida*. (A) The metallic frames, in which seedling strings were wound, were suspended at 1.5 m depth. (B) The seedlings produced by nursery cultivation were stretched horizontally and cultivated on ropes in the *Undaria* farm.

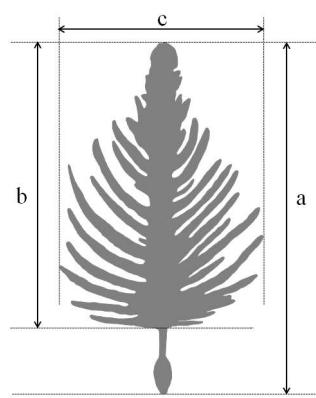


Fig. 3. Illustration of an *Undaria pinnatifida* sporophyte showing total length (a), blade length (b), and blade width (c).

temperature of 24 °C and to harvest adult sporophytes early, resulting in significant benefits (Niwa and Harada, 2016). Thereafter, gametophyte strains have been isolated from wild and cultivated sporophytes as breeding materials in an attempt to produce a superior cultivar with both a higher yield and higher temperature tolerance. From preliminary field-experiments, the cultivar NW-1 was regarded as the most superior among cultivars produced through crossbreeding. To evaluate whether

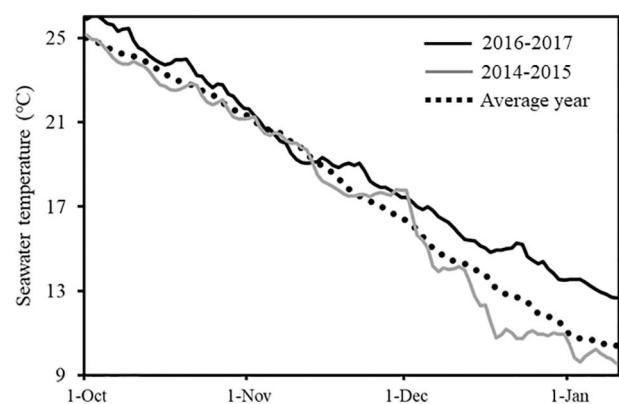


Fig. 4. Change in the mean daily seawater temperature near the cultivation site from October 1 to January 10 in this experimental year 2016–2017 (solid line), the mean daily seawater temperature for the previous experimental year of 2014–2015 for the cultivar HGU-1 (gray line), and the seawater temperature averaged for each year over the past decade 2006–2015 (dotted line).

the cultivar NW-1 is superior to the cultivar HGU-1 developed in a previous study (Niwa and Harada, 2016), this study compares the growth characteristics throughout the experimental cultivation of the two cultivars under high seawater temperatures.

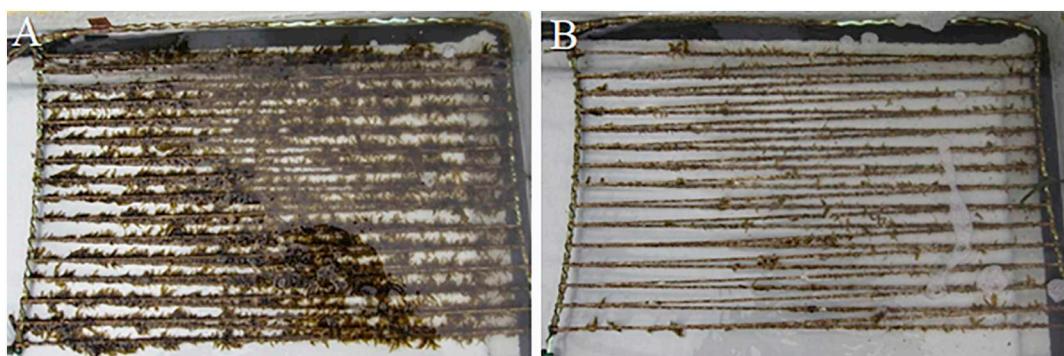


Fig. 5. The metallic frames during nursery cultivation on October 21, 2016. (A) NW-1. (B) HGU-1.

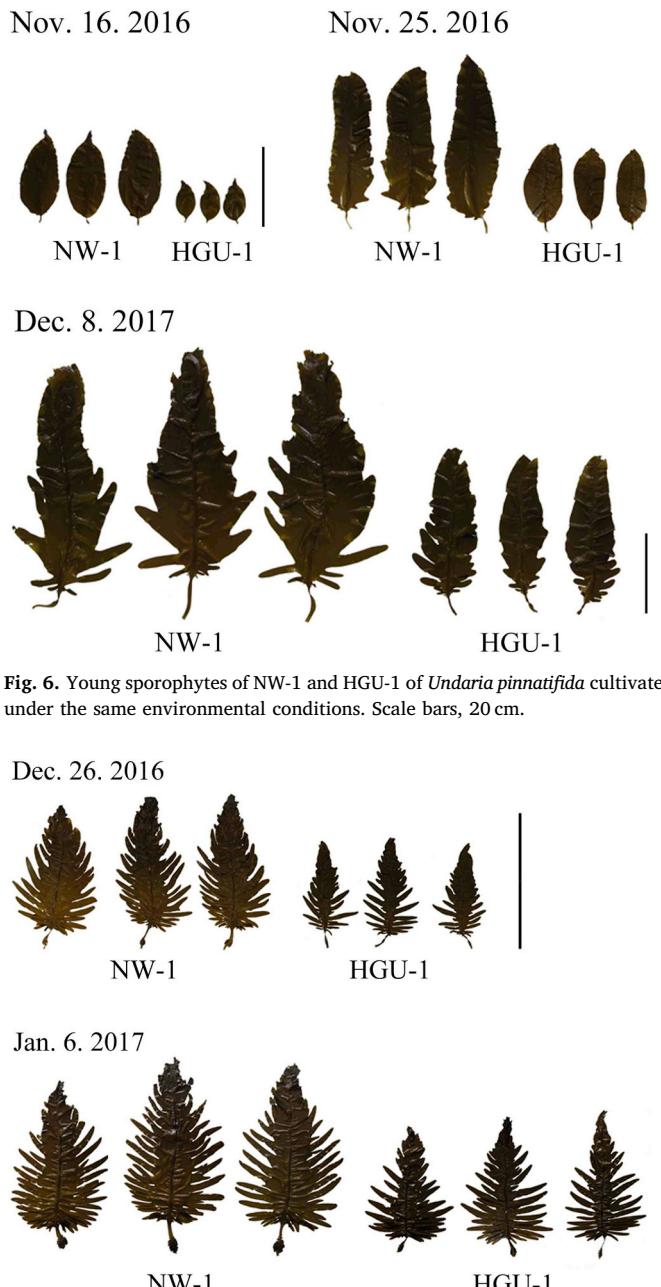


Fig. 6. Young sporophytes of NW-1 and HGU-1 of *Undaria pinnatifida* cultivated under the same environmental conditions. Scale bars, 20 cm.

2. Materials and methods

Mature sporophytes of two different cultivars, called “Shibahara-dane” and “Maruyama-dane” by *Undaria* cultivators, were collected from *Undaria* farms at Maruyama, Awaji Island in Hyogo Prefecture, Japan. The male gametophyte strain (Shibahara-2) and female gametophyte strain (Maruyama-1) were isolated from the sporophytes of “Shibahara-dane” and “Maruyama-dane”, respectively, and were used as parental strains for the breeding of NW-1. The two strains were established from a single zoospore released from each of the two sporophytes, following the procedure described by Niwa (2015) and Niwa et al. (2017). The cultivar NW-1 was produced by cross-fertilization between the male gametophyte strain (Shibahara-2) and the female gametophyte strain (Maruyama-1). In this study, the cultivar HGU-1 developed for forcing cultivation (Niwa and Harada, 2016) was also used for growth comparisons. Seedling production was started on September 7, 2016, using free-living male and female gametophytes and a combined culture in a flat vessel and indoor water tank (Niwa and Harada, 2016). After indoor culture for approximately 1 month, the germlings (juvenile sporophytes) of NW-1 and HGU-1 were transferred to the sea for nursery cultivation on October 11, 2016, in an *Undaria* farm at Eigashima, Akashi, Hyogo Prefecture (Fig. 1). The nursery (Fig. 2A) was conducted according to the procedure outlined in Niwa (2016). After nursery, the seedling strings were cut and inserted into the ropes at approximately 50 cm intervals. The strings of NW-1 were inserted on October 30, 2016. However, since most of the HGU-1 germlings fell from the strings during the nursery and growth of the remaining germlings was remarkably slower, the seedling strings of HGU-1 were inserted on November 6, 2016. The seedlings were immediately cultivated on the ropes stretched horizontally in the floating cultivation facility of the farm (Fig. 2B). The cultivated sporophytes were periodically collected from November 16, 2016 to January 6, 2017, and the total length, blade length, and blade width were measured from the longest ten individuals (Fig. 3). In addition, the total wet weight and blade wet weight were also determined from the collected samples. The measurement data was statistically tested using the Student's *t*-test. When standard deviation (SD) was significantly different, a Mann-Whitney *U* test was applied. GraphPad Prism 7 was the software used for all statistical analysis. Mean daily seawater temperature was detected by an automatic measuring device fixed near the Fisheries Technology Institute, Hyogo Prefectural Technology Center for Agriculture, Forestry and Fisheries, Akashi, Hyogo Prefecture (see Fig. 1).

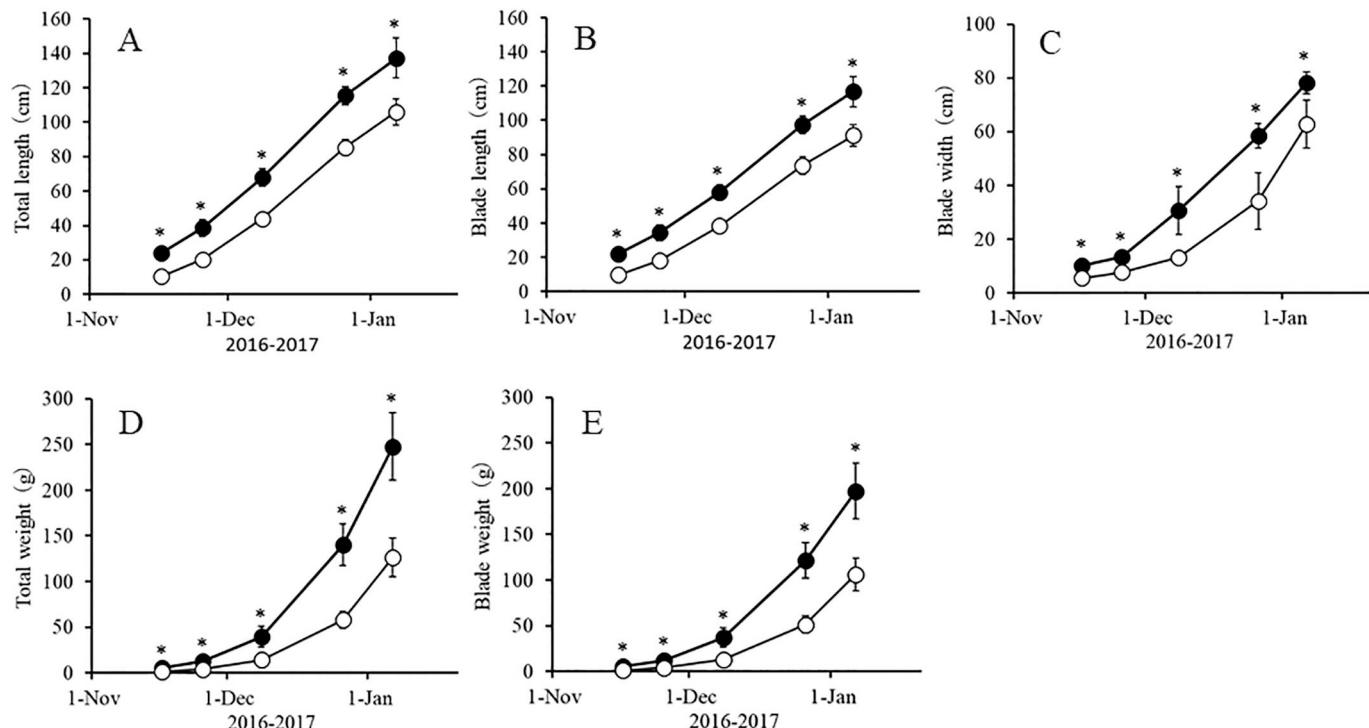
3. Results

Fig. 4 shows the change in the mean daily seawater temperature near the cultivation site from October 1 to January 10 (2016–2017), the mean daily temperature of the previous experimental year for HGU-1 cultivation (2014–2015), and an average yearly temperature for the past decade (2006–2015). During the 2016–2017 trial cultivation, the

Fig. 7. Adult sporophytes of NW-1 and HGU-1 of *Undaria pinnatifida* cultivated under the same environmental conditions. Scale bars, 1 m.

Table 1Comparison of size characteristics between NW-1 and HGU-1 sporophytes of *Undaria pinnatifida* collected on December 26, 2016, and January 6, 2017.

	Dec. 26, 2016		Jan. 6, 2017	
	NW-1	HGU-1	NW-1	HGU-1
Total length (cm)	111.5 ± 5.3*	85.4 ± 4.5	137.3 ± 11.6*	106.0 ± 7.6
Blade length (cm)	97.4 ± 5.1*	73.6 ± 5.1	116.7 ± 8.9*	91.2 ± 6.3
Blade width (cm)	58.5 ± 4.6*	34.2 ± 10.4	78.2 ± 4.0*	62.9 ± 8.9
Total weight (g)	140.1 ± 22.5*	58.1 ± 9.4	247.6 ± 36.9*	126.6 ± 21.1
Blade weight (g)	121.7 ± 19.5*	51.3 ± 9.3	197.5 ± 30.7*	106.3 ± 18.0

Average ± SD (n = 10). Asterisks indicate significant difference between NW-1 and HGU-1 ($p < .0001$).**Fig. 8.** Growth of NW-1 and HGU-1 sporophytes of *Undaria pinnatifida*. (A) Total length. (B) Blade length. (C) Blade width. (D) Total weight. (E) Blade weight. Average ± SD (n = 10). Asterisks indicate significant difference between NW-1 and HGU-1 ($p < .0005$).

seawater temperature was higher than that of an average year, except for early November. In particular, the seawater temperature from December to early January was two to three degrees higher in this experimental year than in an average year.

In this study, nursery started at the *Undaria* farm on October 11, 2016. The mean daily seawater temperature at the start date was 24.5 °C. On October 21, many young sporophytes of NW-1 grew wholly on the strings wound around the collector (Fig. 5A), whereas most of the HGU-1 sporophytes fell from the strings (Fig. 5B). In addition, the remaining HGU-1 sporophytes were remarkably smaller than those of NW-1.

The young NW-1 and HGU-1 sporophytes that were stretched horizontally to be cultivated on ropes are shown in Fig. 6. The sporophytes of NW-1 were remarkably larger than those of HGU-1 on November 16, November 25, and December 8. The mean total lengths on November 16, November 25, and December 8 were 24.0, 38.7, and 67.8 cm for NW-1 and 10.4, 20.3, and 43.9 cm for HGU-1, respectively.

Fig. 7 shows the adult NW-1 and HGU-1 sporophytes on December 26 and January 6 prior to harvesting, the measurement data of which are summarized in Table 1. These sporophytes were also remarkably larger in NW-1 than in HGU-1. The wrinkles on the blade portion adjacent to the midrib were observed in both cultivars, but they were slight in case of NW-1. All NW-1 sporophytes measured on December 26

formed sporophyll. On the other hand, only one of the HGU-1 sporophytes measured at the corresponding time formed sporophyll. Fig. 8 shows growth comparisons between NW-1 and HGU-1 throughout cultivation. As shown in Figs. 6 and 7, total length, blade length, blade width, total weight, and blade weight of NW-1 were significantly higher than those of HGU-1 during cultivation.

4. Discussion

Nursery cultivation for *U. pinnatifida* is usually started when seawater temperatures are 23 °C in the eastern Seto Inland Sea (Dan et al., 2015). The cultivation was started at 24.5 °C, which is 1.5 °C higher than usual. Despite this, many juvenile sporophytes of NW-1 grew entirely on the strings of the collectors. When HGU-1 was cultivated previously at 24 °C, many seedlings were produced (Niwa and Harada, 2016); however, most of the HGU-1 sporophytes quickly fell from the strings when cultivated at 24.5 °C. This result indicates that compared to HGU-1, NW-1 has a higher temperature tolerance which enables *Undaria* cultivators to start the cultivation at higher seawater temperatures.

Although the total length of the *Undaria* sporophyte, cultivated in the eastern Seto Inland Sea at the end of December, is usually about 50 cm at the most, the HGU-1 sporophyte cultivated by forcing

cultivation grew to 105.0 cm in mean length by December 26, 2014 (Niwa and Harada, 2016). During 2014–2015, seawater temperature in December was lower than that of the average year. On the other hand, since seawater temperature after starting rope cultivation in this study was remarkably higher compared to that of the average year, the mean total length of HGU-1 on December 26, 2016, was 85.4 cm, which is shorter than the previous study (Niwa and Harada, 2016). However, the NW-1 seedlings developed into sporophytes with a mean length of 115.5 cm on December 26, 2016, despite a high seawater temperature. Furthermore, the NW-1 sporophyte was significantly larger and heavier than the HGU-1 sporophyte throughout this experiment period. These indicated that NW-1 is an excellent cultivar with a high yield and high-temperature tolerance.

The seedlings of *U. pinnatifida* in Japan are usually produced yearly from zoospores released from sporophylls cut from many sporophytes, and this method cannot enable the seedling production implemented with the same genotype every year. In contrast, seedling production using free-living male and female gametophytes, each isolated from a single zoospore, can produce genetically identical seedlings every year. Because it could maintain these gametophytes in laboratory culture for a long time, the seedling production technique using free-living gametophytes is essential for NW-1 cultivation. However, due to the laborious procedure, almost all the cultivators in Japan do not produce the seedling using free-living gametophytes. Recently, a simple and practical method of seedling production has been developed for forcing cultivation in *U. pinnatifida* (Niwa and Harada, 2016). Furthermore, this mass seedling production using large indoor tanks has also been developed for stable and high-quality production of this marine crop (Niwa, 2016). The cultivators have cultured parental gametophytes of NW-1 using incubators and produced NW-1 seedlings through these seedling production for three years, and regard NW-1 as an excellent cultivar. In particular, those in Maruyama, Awaji Island trust the high productivity and quality of the cultivar NW-1.

The gametophytic phase of *U. pinnatifida* is dioecious, and it is easy to cross between the genetically different strains. Hara and Akiyama (1985) reported that the F₁ hybrid of the marine crop shows heterosis. Thus, the crossbreeding has been attempted as a practical method for the development of superior cultivars including breeding of cultivars with high-temperature resistance (Pang et al., 1997; Kato et al., 2010; Tanada et al., 2015; Niwa and Harada, 2016; Shan et al., 2016). Recently, breaching of *U. pinnatifida* sporophytes is often caused by nitrogen depletion in the eastern Seto Inland Sea during the latter cultivation stage. It is necessary to start the cultivation early, even though economic loss by grazing of herbivorous fish often occurs in the early period of *Undaria* cultivation (Kiriyama et al., 2000). Here our developed cultivar will be positive to reduce economic damage caused by bleaching of the marine crop.

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